

# TECHNIQUES FOR RAISING PENAEID SHRIMP FROM THE EGG TO POSTLARVAE<sup>1</sup>

Cornelius R. Mock and M. Alice Murphy  
Bureau of Commercial Fisheries Biological Laboratory  
Galveston, Texas 77550

## ABSTRACT

Techniques for raising penaeid shrimp from the egg to post-larvae are discussed. Equipment, rearing methods, algal culture, collection of gravid females, spawning procedures, larval culture, and harvesting techniques are described step by step.

## INTRODUCTION

Shrimp mariculture research at the Bureau of Commercial Fisheries Biological Laboratory, Galveston, Texas has reached the point where penaeid shrimp can be reared regularly from the egg to postlarvae in the laboratory. The initial work at the Galveston Laboratory was done by Cook and Murphy (1966, 1969) who described in detail the equipment and techniques necessary to culture relatively large numbers of penaeid shrimp to the postlarval stage.

The new procedures and equipment described in this paper were designed to increase our hatchery potential for pond stocking. We were interested not only in raising more shrimp, but also in designing a hatchery that would be more efficient and economical. For example, electrical pumps were used previously throughout the operation to transport diatoms and to recirculate water in each rearing tank. Because electricity is expensive and involves a safety factor, construction of a diatom-culture room above the hatchery enabled us to feed by gravity flow. Also, by designing a recirculating filter chamber for each rearing tank, that utilizes compressed air instead of electricity, we were again able to eliminate electrical pumps.

This paper is an up-to-date report of equipment and procedures used to rear shrimp to the postlarval stage. With these modifications we have increased our postlarval yield to the point where pond stocking is now possible.

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## EQUIPMENT

## Algal Culture Equipment

Due to the variable chemical characteristics of natural seawater, algal cultures are now grown in synthetic sea salt, Instant Ocean,<sup>2</sup> and tap water. All water used throughout the experiment is passed through a 5 $\mu$  filter. The amount of salt added is predetermined for each species of diatom. Algal cultures are grown in rectangular polyethylene tanks (200 l or 400 l capacity) in a temperature-controlled light room above the shrimp hatchery. Banks of fluorescent lamps (F72T12/CW/HO), which can be lowered or raised according to the amount of light required, are suspended above the tanks (Figure 1).

Constant mixing of the culture is accomplished by air flow through a series of four to six air stones distributed throughout each tank. When the diatom *Thalassiosira* sp. was grown in early experiments, it settled to the bottom and accumulated in the corners even though the culture was being mixed. This was corrected by placing plastic cylinders (2.5 cm in diameter and 16 cm long) with an air stone in the corner of each tank. Air bubbles flowing up this cylinder created a water lift and prevented accumulation of diatoms in the corners of the tank. This system is now being used in all algal culture tanks.

## Conditioning, Spawning, and Rearing Containers

Conditioning tanks may be of any size depending on the number of gravid female shrimp captured. Either plastic or fiberglass tanks are suitable.

The spawning tanks are inverted lg 1 plastic carboys with the bottoms cut out. The mouths of the carboys are plugged with rubber stoppers through which air hoses are inserted.

Rearing tanks are round and range in capacity from 946 l to 1,892 l. They are constructed of fiberglass or polyethylene, and the bottom may be flat or cone-shaped. Water is aerated by lead-weighted air stones suspended from the sides of each tank.

## Filtering Systems

The water is filtered through built-in filters (Figure 2) that are operated by air bubbled at a rate of 1 l per minute and are screwed into the bottom of each tank. Four holes, 4.4 cm in diameter, are made along the bottom of the filter. A wooden disk (1.3 by 15.2 cm) with a 3.8 cm hole drilled in the center

is glued to the bottom of the chamber. Another wooden disk (6 by 15.2 cm), with a 10.2 cm hole drilled in the center is positioned 30.5 cm below the top of the filter and glued to the pipe. All wooden parts are painted with an epoxy paint. A nylon sleeve, 0.13 mm mesh, is then stretched between the two wooden disks and glued to them. A 5.1 cm bulkhead fitting is secured to the bottom disk and a nipple is attached.

The air-lift mechanism is constructed from a plastic pipe 53.3 cm long and 3.8 cm in diameter attached to a 5.1 cm "P" trap. A portion of the trap is cut out (Figure 2) to prevent air from accumulating in the top of the trap and restricting the flow of water. An air stone is attached 2.5 cm above the bottom of the 5.1 cm pipe.

Operation and maintenance of the filter chamber are accomplished as follows:

- 1) A perforated plastic funnel is glued to the bulkhead fitting to aid in fitting the filter nipple into the bulkhead fitting.
- 2) Once the filter is screwed into the bottom of the rearing tank, a stiff, perforated plastic sleeve (12.7 cm long, 7.6 cm in diameter, and 1.3 cm mesh) is inserted into the filter chamber.
- 3) A bag of pullet-size crushed oyster shell is placed in the filter and lowered by a string to rest on the sleeve. This bag contains 1.4 l of shell and spreads to the sides of the filter when it reaches the sleeve.
- 4) The 5 cm pipe with the "P" trap is inserted into the filter with the trap extending over the lip of the filter.
- 5) Water flows through the screen, down to the intake openings, through the shell, and up the chamber until it reaches the level of the water in the rearing tank.
- 6) When the air is turned on, it flows up the plastic pipe and lifts water out of the filter and back into the rearing tank. The water is filtered and aerated.
- 7) The filter is cleaned by lifting the plastic pipe out, removing the bag of shell, and replacing it with a clean one. The pipe is inserted and the filter is once again ready to operate.

We have also built a filter which operates outside the rearing tank (Figure 3). The filter screen and shell filter are separate units. The filter screen is made of a nylon sleeve (0.13 mm mesh, 15 cm in diameter, and 61 cm long) and is attached to a 5 cm PVC pipe threaded at one end. The PVC pipe extends above the level of the water when in place. Plastic supports inside the sleeve keep it rigid. The portion of the pipe covered by the sleeve is perforated by numerous holes 1.9 cm in diameter.

<sup>2</sup>Trade names referred to in this publication do not imply endorsement of commercial products.



The shell filter is constructed of 10 cm plastic pipe and stands alongside the rearing tank. The bottom of the chamber is sealed with a wooden plate. About 5 cm above the bottom of the chamber, a 5 mm air line and 2.5 cm hose fitting are installed. An arrangement of fittings and valves connects the rearing tank with the shell filter and drain (Figure 3). A total of 19 l of oyster shells are placed inside the shell filter. A perforated plastic screen (1.3 cm mesh) fixed 15.2 cm above the bottom of the chamber supports the shell in the chamber. A plastic pipe with a "P" trap attached as described previously is placed in the top of the filter. The "P" trap extends over the edge of the rearing tank. This filter operates in the same way as the filter that fits inside the rearing tank. This filter is cleaned as follows: 1) The inline valve is closed. 2) Air to the plastic pipe is turned off and the air to the bottom of the chamber is turned on. Bubbles forced through the shell dislodge foreign material which is flushed from the filter by water directed into the top of the filter. Excess water flows through the drain hole in the top of the filter. 3) The drain valve is opened and the filter is back-flushed.

#### REARING METHODS

##### Algal Culture

It is necessary to start the algal culture several days before gravid female shrimp are captured because it takes several days to bring the cultures up to a suitable level for feeding.

The mass culture (200 l) of the diatom, *Skeletonema* sp. which was initially begun from a 7.6 l inoculation, was maintained in tap water and Instant Ocean adjusted to a salinity of 26 ppt. Additives used are listed in Table 1. The temperature of the culture was maintained between 25 and 30 C, and illumination was at an intensity of 8,074 - 10,765 lux. The culture was maintained for 10 days, and densities of 8.0 to 10.0 x 10<sup>6</sup> cells per ml were obtained. A dense culture survived for an additional 4 days with no harvesting and no additions of nutrients.

##### Collection of Females

Because we are unable to rear female shrimp to sexual maturity within the laboratory, we collect gravid female shrimp offshore and bring them to the laboratory for spawning. Shrimp are collected with standard shrimp fishing gear towed for 10-20 minutes. Once the gravid females are captured, they are placed in ice chests containing 30 l of seawater. The ice chests are equipped with portable aerators, and no more than 24 shrimp are held in each container. To avoid mortality during warm weather, the water temperature is reduced to about 24 C by placing plastic bags of ice in the ice chests. A portion of the water in each chest is drained off and replaced with fresh seawater every 2 or 3 hours.

The day the boat goes out to search for gravid female shrimp, the conditioning, spawning, and rearing tanks are filled with seawater and the salinity is adjusted to 30 ppt by adding either synthetic sea salt or tap water.

#### Conditioning Procedures

When female shrimp spawn in water less than 24 C, the survival of the nauplii is poor (Cook and Murphy, 1969). If the temperature of the water in the ice chests is maintained above 24 C, the temperature conditioning is omitted; however, if the water temperature is lower than 24 C, the shrimp are conditioned to a higher temperature. An electrically controlled immersion heater is used to raise the water temperature to 26 C. From this point on, all water that the shrimp come in contact with contains the sodium salt of EDTA [(Ethylenedinitrilo)-tetracetic acid], at a concentration of 0.01 g per l of water. Transfer of the shrimp from the conditioning tanks to the spawning carboys should be completed by 7:00 p.m. on the day shrimp are brought into the laboratory because they usually spawn the first evening.

#### Spawning Procedures

Each gravid female shrimp is placed in a spawning carboy, and the flow of air is greatly reduced. Netting is stretched across the carboys to prevent the shrimp from jumping out. Visual checks for eggs are made throughout the evening. When a shrimp spawns, she is removed from the carboy, and the total contents of the carboy are poured into the rearing tank.

#### Larval Culture

Daily observations of the larvae in the rearing tanks are made to ascertain stages of larval development. This is particularly important during the naupliar stages. When the larvae reach the fifth naupliar stage, algae are added so that food will be available when the larvae become protozoae. Algae are fed to the protozoal stages only, and freshly hatched brine shrimp (*Artemia* sp.) are fed to the mysis and postlarval stages. Algal cells are counted with a hemacytometer; brine shrimp are counted with a mechanical nauplii counter. The amount of food fed is governed by: 1) number of larvae present, 2) stage of larval development, and 3) general condition of the larvae such as, the presence of food in the gut, the presence of fecal chains, and the color of the larvae.

#### Aliquot Counts

The number of shrimp in each tank is determined by making aliquot counts when the shrimp are in each stage of development, i.e., naupliar, protozoal, mysis, and postlarval. A glass cylinder, 12.7 cm long and 1.9 cm in diameter, is inserted vertically through the column of water in the rearing tank and closed at the top, thus



capturing the larvae in a known volume of water. The cylinder and its contents are then removed from the tank, and the water is drained into a graduated beaker.

Naupliar stages I and II are counted by pouring the contents of the graduated beaker through a mechanical counter. Larvae of the more advanced stages are concentrated, and a sample placed in a petri dish. A polaroid picture is taken of the petri dish and contents, and the picture is examined through a dissecting microscope. Not only do the pictures reveal the number of larvae, but orientation of the larvae and coloration denote if they are dead or alive. The data from the aliquots are then fed into a computer, and the mean, standard deviation, and confidence limits of the estimate of total shrimp per volume of water are determined.

#### Harvesting Techniques

The harvesting container (Figure 4) is composed of a round tank (48.3 cm in diameter and 40.6 cm tall) inside a square tank (60.9 cm by 60.9 cm by 30.5 cm). Both are plastic. Four portholes, 11.4 cm in diameter and covered with a 0.13 mm screen, are along the side of the round tank. Two standpipes are affixed to the square tank. When water is placed in the round tank, it is filtered through the screen into the square tank. The level of water desired in the tanks can be controlled by lowering or raising the standpipes. Before transferring the shrimp to the harvesting container, the container is filled with seawater that is forced into a swirling motion. This swirling motion is desirable because it causes the shrimp to accumulate in the center of the tank rather than on the porthole screens. The plastic hose from the rearing tank is directed so that the incoming water maintains the swirling motion.

Harvesting and counting of the postlarvae in the rearing tank are performed in the following manner: 1) With the filter in place, the drain valve to the rearing tank is opened and the volume of water is reduced to about 189 l, thus concentrating the postlarvae. 2) The drain valve is closed and the filter is removed. 3) A 5 cm plastic hose is connected to the drain to relay the shrimp to the harvesting container. 4) After the transfer is completed, the swirling is stopped and water is adjusted to a desired volume. 5) The water is then mixed to distribute the shrimp randomly, and aliquots are taken and shrimp are counted in the manner described previously.

#### A REARING EXPERIMENT

On November 5, 1969, a number of gravid female brown shrimp (*Penaeus aztecus*) spawned in the laboratory. Each gravid shrimp was held in a separate spawning container until she had spawned. The contents of two of the spawning carboys were poured into one of the rearing tanks (946 l). Using this procedure, we distributed the eggs equally among the rearing tanks. This procedure also

prevents the female from eating the eggs.

Examination of Figure 5 reveals those parameters measured throughout a successful rearing experiment. Of particular interest are the periodic counts of the diatoms in the rearing tank; these indicate the number of cells eaten by the larval shrimp. As grazing increased the level of feeding was increased.

Aliquot counts of the number of larvae revealed that 83% of the population survived to protozoa III and that 75% reached the postlarval stage. Apparently diatom levels were not high enough in the larval cultures at certain times during the experiment, i.e., when counts were less than 100,000 cells per ml. Although only one rearing tank is discussed here, shrimp were actually reared in five other rearing tanks at the same time.

In previous rearing experiments, diatoms were frequently grown in natural sea water. Samples of the sea water were tested with several types of concentrations of nutrient salts to determine the best combination for diatom growth. Even when this procedure was used, dense growth did not always result, and in many cases rapid growth of the culture could not be maintained for more than 3 to 5 days. Also, because of the low culture densities of diatoms, a large amount of space was required for the culture, and large volumes of the culture medium had to be transferred to the rearing tanks. The result of this largescale transfer was that the quality of the larval culture medium could not be maintained and had to be adjusted daily. These problems were eliminated by developing techniques for culturing the diatoms in a more dependable medium consisting of tap water and Instant Ocean sea salt.

When shrimp became postlarvae, they were removed from the culture tanks and transferred to other facilities or shipped to other research agencies for experimental studies. Postlarvae were shipped by air in styrofoam-lined cardboard boxes (Figure 6). Shrimp were held in sealed plastic bags filled half with water and half with oxygen. By this method, as many as 10,000 postlarvae 6 mm in length can be held in 13 l of water for 24 hours or more without distress.

#### LITERATURE CITED

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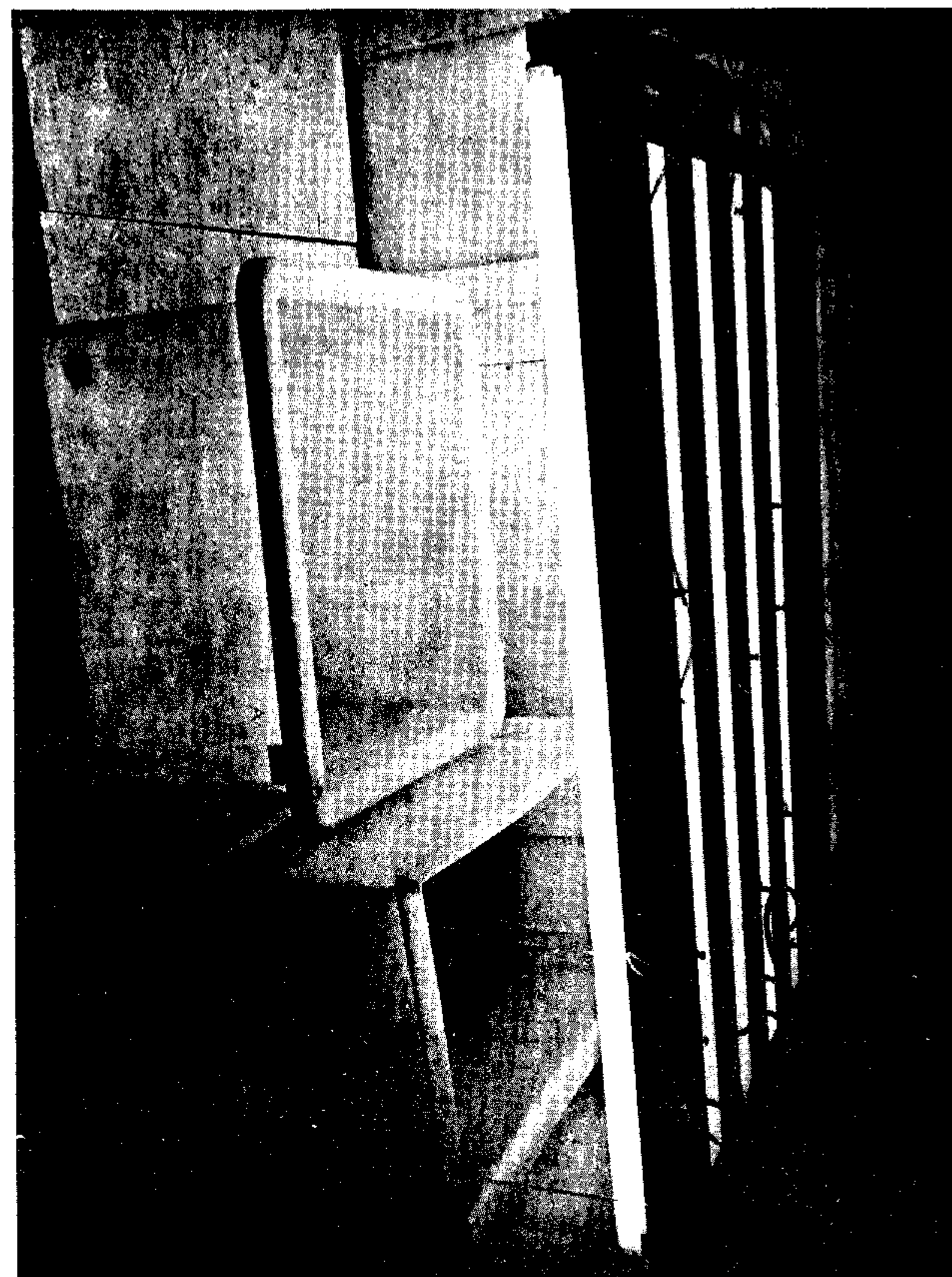


Table 1. - Additives to the medium used  
to culture Skeletonema sp.

Additive	Concentration of solution	Volume of solution added per liter of medium <sup>1</sup>
Tris buffer (2-amino- 2 (hydroxymethyl)-1, 2-propanediol	100 g /l	4.0 ml (after buffering to pH 8.3)
Vitamin B <sub>12</sub>	1.0 µg /l	1.0 ml
Thiamine	10 mg /l	10.0 ml
NaSiO <sub>3</sub>	10 g /l	1.1 ml
FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub>	10 g /l	0.99 ml
KNO <sub>3</sub>	10 g /l	10.0 ml
EDTA	10 g /l	1.0 ml

<sup>1</sup>Tris buffer is added to initial culture medium and to new medium added each day. All other additives are added to total volume of culture medium each day.

Figure 1. Polyethylene tanks and light bank used for algal cultures.



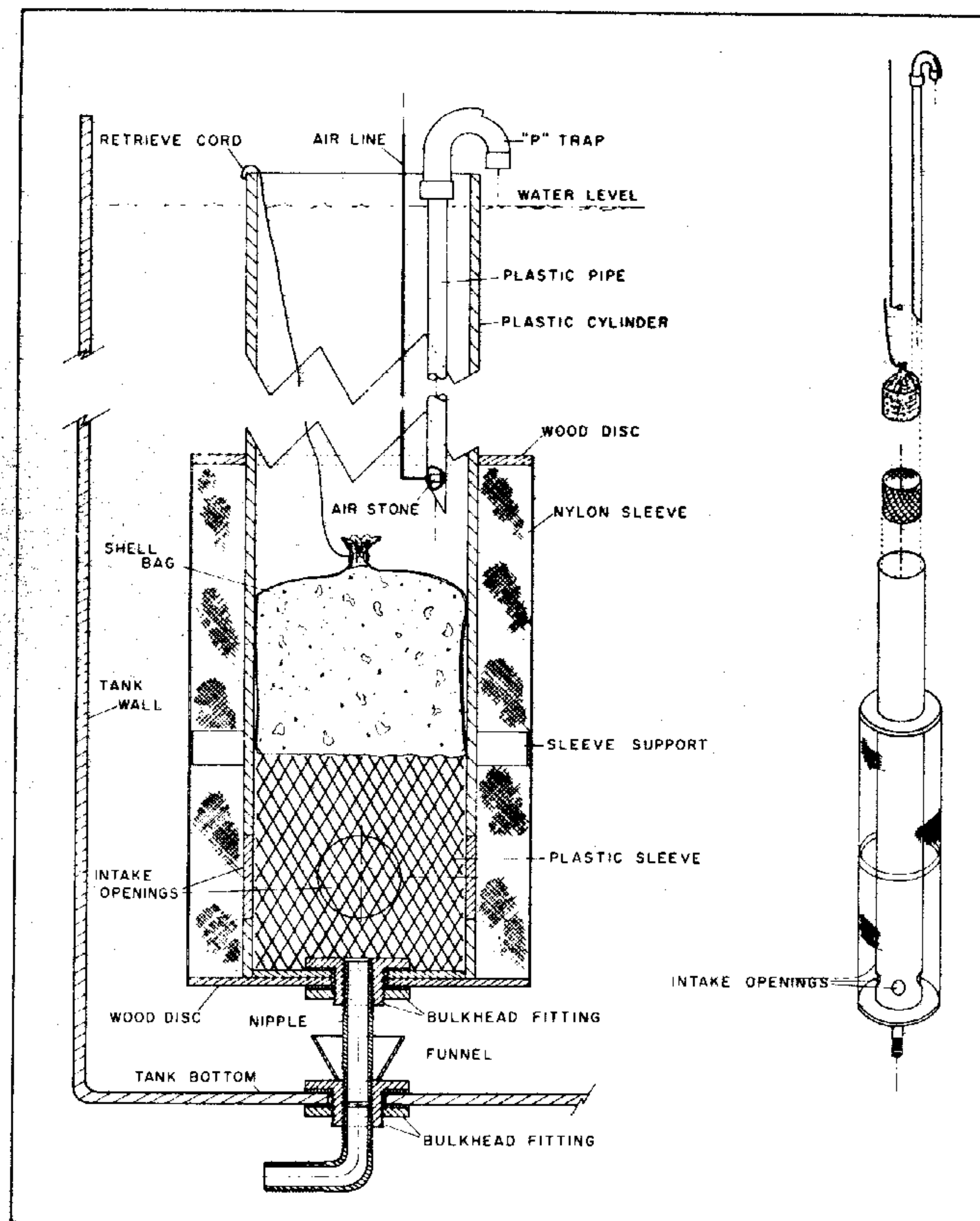


Figure 2. Air-operated filter built into the rearing tank. Water flows from the rearing tank through the nylon sleeve, through the intake openings, up through the oyster shell, and through the plastic pipe back into the tank.

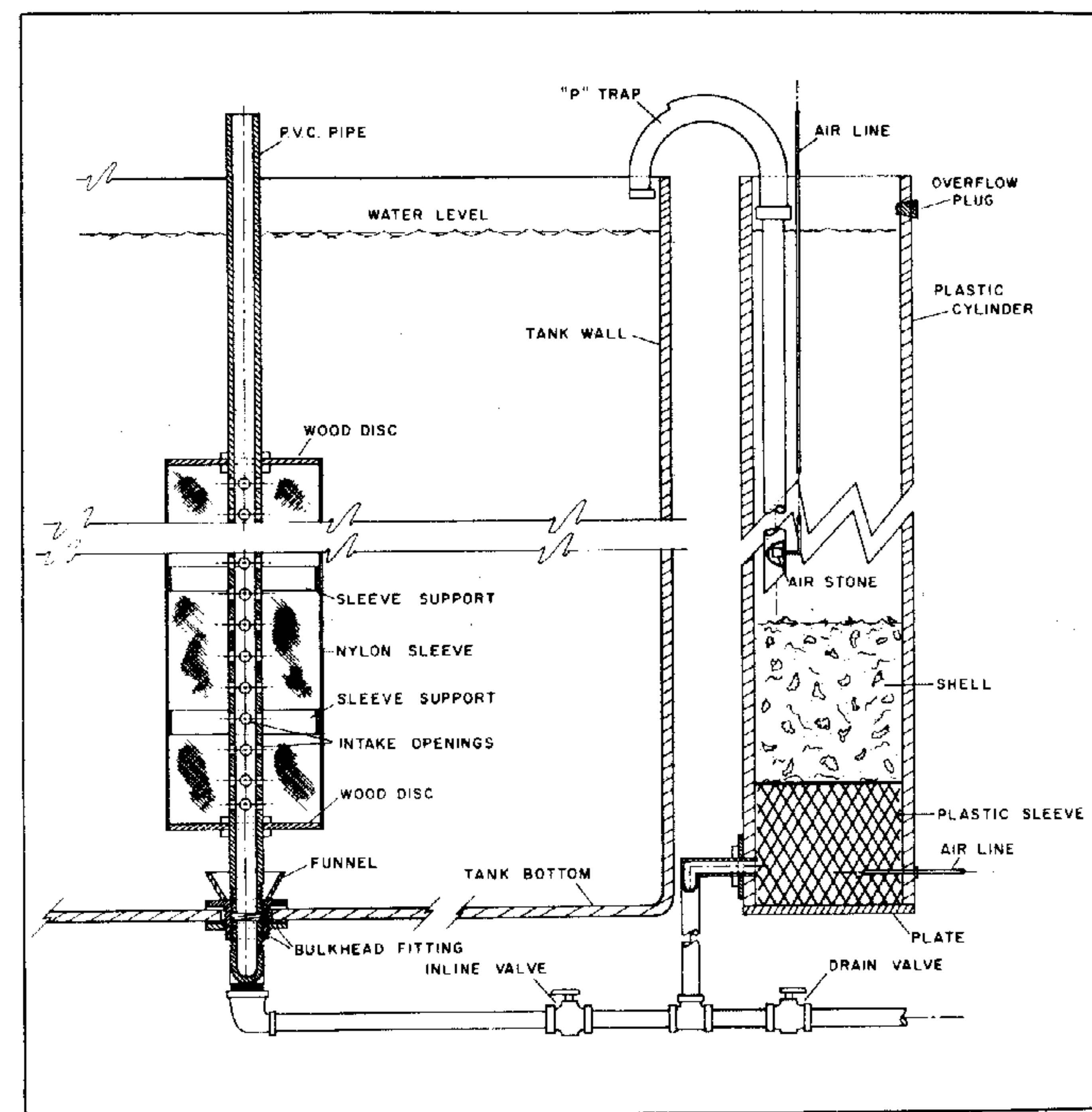


Figure 3. Filter system with outside shell filter.



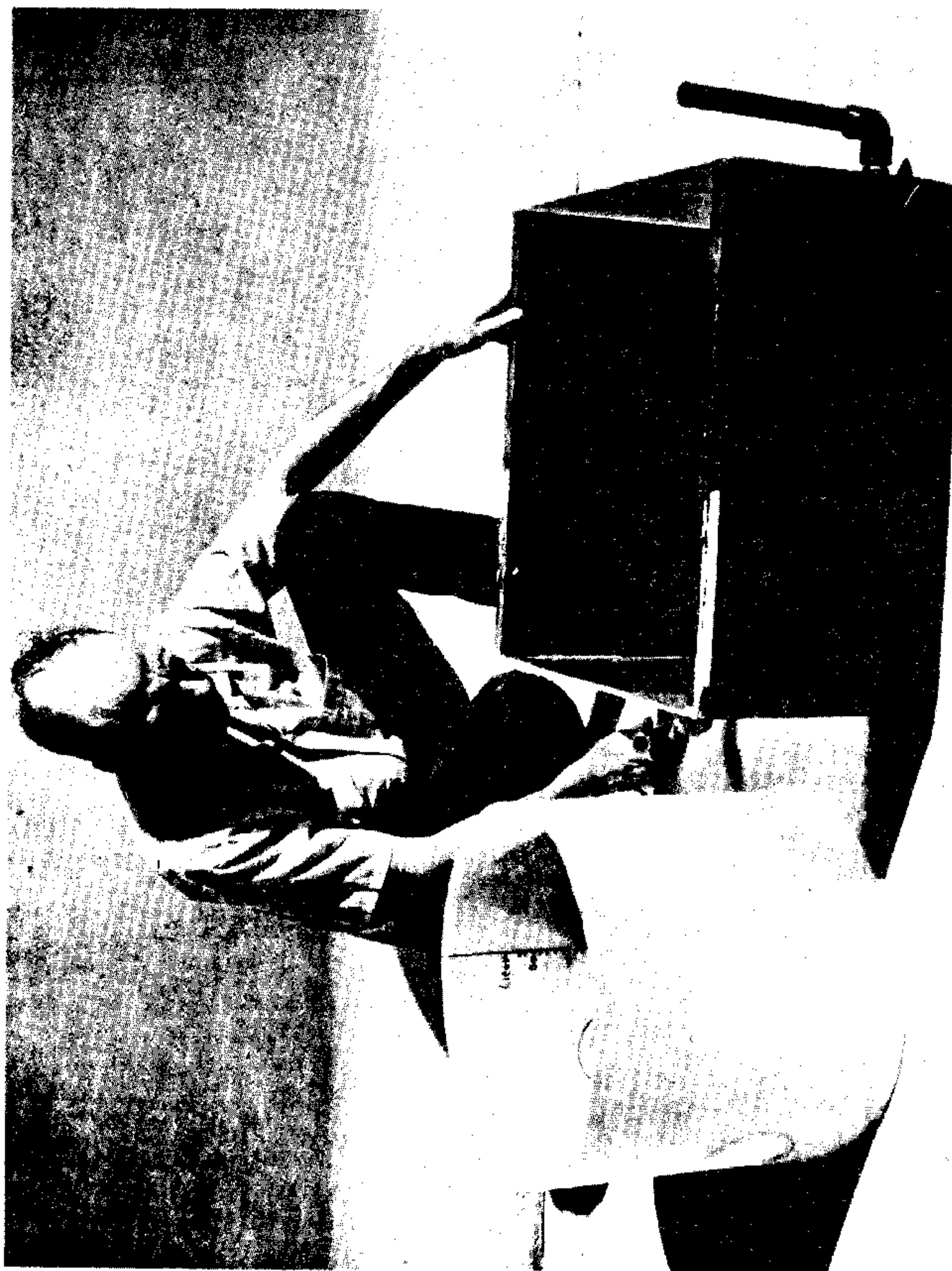


Figure 4. Harvesting container used to concentrate the contents of the rearing tanks for population density estimates.

Figure 5. Utilization of diatoms by brown shrimp protozoae and *Artemia* by brown shrimp mysids and postlarvae over a 9-day period.

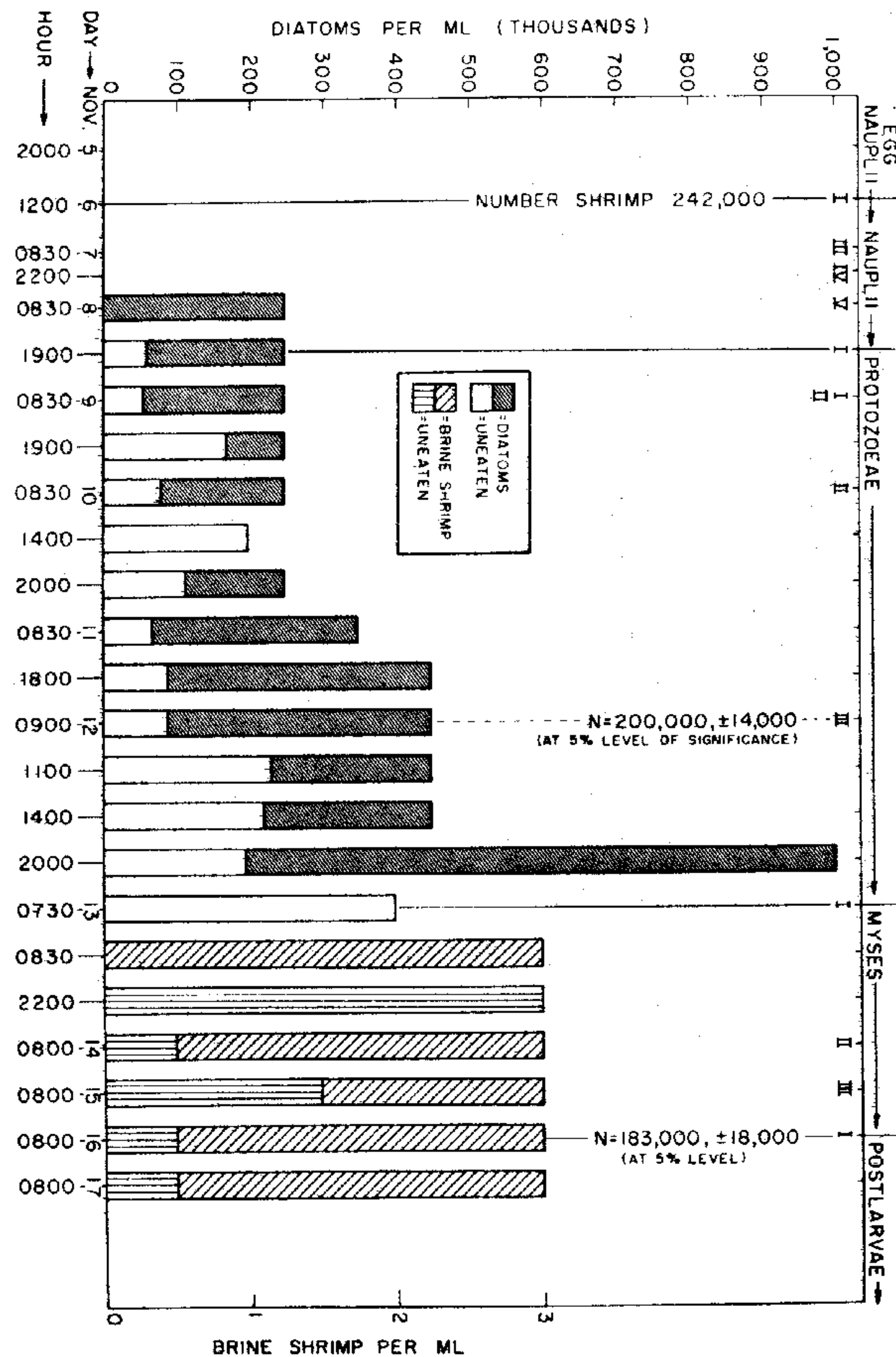




Figure 6. Containers used to ship postlarval shrimp.